CHAPTER V A

EFFECTS OF DRUGS ON THE REPRODUCTIVE PHYSIOLOGY OF MALE ALBINO RATS

1. TRANQUILIZERS AND SEDATIVES

INTRODUCTION

A number of tranquilizers and sedatives are known to affect the reproductive organs either directly or indirectly through the pituitary-gonadal axis and central nervous system (Yamazaki and Nakayama, 1972; Saldanha et al., 1972; Levitt, 1975; Satoskar and Bhandarkar, 1975), so that gonadal steroidogenesis is impaired concomitant with reduced testicular androgenicity, sperm metabolism and secretory activity of the accessory reproductive glands in male rats and ovulation block in females (Beattie et al., 1973; Sindgi and Rao, 1975; Rao and Sindgi, 1976; Rao, personal communication; Chinoy and Sheth, 1976; Buch, 1976). On the contrary, some reports indicate that the barbiturates do not inhibit LH release or affect testicular physiology (Wedig and Gay, 1973; Stripp et al., 1974; Purandare et al., 1975).

Administration of a variety of drugs including sedatives and tranquilizers lead to increased synthesis and utilization of L-ascorbic acid (Longenecker et al., 1940; Mckernan and Teague, 1973; Chinoy and Sheth, 1976) for the detoxification or biological inactivation of histamine (Subramanian et al., 1973;
1974; Nandi et al., 1974). The tranquilizer/sedative induced alterations in sperm metabolism and accessory gland functions were found to be reversible by the administration of ascorbic acid along with the drug (Chinoy and Sheth, 1976; Buch, 1976). The beneficial effects of ascorbic acid were mediated by the enhanced formation of its free radical monodehydroascorbic acid (Buch, 1976). Moreover, the metabolism of testis and epididymis of vitamin C deficient, drug treated guinea pigs were found to be more affected than those animals which were fed a vitamin C fortified diet (Chapter VI A), as scorbutic guinea pigs are unable to metabolize the drug efficiently (Garattini and Shore, 1962). A reversal of the decreased microsomal drug metabolizing enzyme activities in vitamin C deficient guinea pigs was observed by the administration of ascorbic acid (Zannoni et al., 1972).

In the light of these findings it was thought worthwhile to investigate the effects of drugs like chlorpromazine (tranquilizer), chlordiazepoxide (a depressant tranquiliser) and butabarbitone (a depressant sedative) on the metabolism of testis and epididymis with special emphasis on the role of ascorbic acid.

MATERIALS AND METHODS

Healthy adult albino rats (Rattus norvegicus) weighing between 200-250 gm were used for the experiments. The animals were maintained on standard feeds (Hindustan Lever Ltd., Bombay)
and supplied water ad libitum in an air conditioned animal
house (26±2°C) with 14 day light hours.

The drugs used were as follows:

a. Chlorpromazine (Targactil) injections, I.P. 1% W/V.,

b. Chlordiazepoxide N.F. 10 mg/tablet, Unique Pharmaceutical
   Laboratories Bombay, Lot. No. 1687.

c. Butabarbital 100 mg/tablet, B.P., May and Baker Ltd.,
   Bombay Lot No. 1495.

Of the three drugs (a) and (b) are known to be very
soluble in water, whereas (c) is sparingly soluble (Satoskar
and Bhandarkar, 1975). Therefore a known quantity of each
drug was dissolved in a known volume of water so as to obtain
a concentration of 1 mg/ml for the tranquilizers and 8 mg/ml
for butobarbitone (sedative) which were found to be the
minimum effective doses. In the case of (b) and (c), the
tablets were first powdered, dissolved in water and the clear
aqueous layer was used for injections.

Mode of administration of the drugs:

The animals were given an intramuscular injection of
tranquilizers and sedative (1 ml/animal) which is the most
appropriate mode of administration of each of these drugs,
since a subcutaneous injection may cause local irritation.
intravenous administration might prove fatal due to a sudden fall in the blood pressure (Satoskar and Bhandarkar, 1975).

The animals were divided into the following groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No of animals used</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact normal control</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
<tr>
<td>II</td>
<td>Chlorpromazine (CPZ) treated rats, 7 and 15 days</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
<tr>
<td>III</td>
<td>CPZ+ascorbic acid treated rats. The animals were treated with CPZ as in group II and in addition were fed ascorbic acid (AA) (100 mg/animal/day) orally for 7 and 15 days</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
<tr>
<td>IV</td>
<td>CPZ + testosterone propionate (TP) treated animals The rats were treated with CPZ as in group II and in addition were given intramuscular injections of TP (200 μg/animal/day) for 7 days</td>
<td>12</td>
<td>8th</td>
</tr>
<tr>
<td>V</td>
<td>Chlordiazepoxide treated rats, 7 and 15 days</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
<tr>
<td>VI</td>
<td>Butobarbitone treated rats 7 and 15 days</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
</tbody>
</table>

The animals were autopsied after the respective treatment and the testis and epididymides (Caput and cauda) were excised, blotted free of blood, cleared of the fat bodies.
and connective tissue and weighed. Thereafter they were used for biochemical studies whose details are given in Chapter I.

Free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complexing (AA-MM): (Chinoy et al., 1974).

Ascorbic acid free radical (AA-FR) forming special peroxidase: (Chinoy, 1973).

Cholesterol: (Pearson et al., 1953).

Succinate dehydrogenase (SDH): (Kun and Abood, 1949).

Alkaline and acid phosphatases: (Belfield and Goldberg, 1971).

Protein: (Gornall et al., 1949).

A minimum of six replicates were done for each tissue and parameter and the results were statistically analyzed using student's 't' test.

RESULTS

Organ weights:

The drug treatment did not alter the weights of testis and epididymides except that a decline (P < 0.02, 0.01) was noted in the weights of caput and cauda epididymides by CPZ (7 and 15 days) and butobarbitone (7 days). A combined treatment with CPZ + TP was more effective than CPZ + AA in recovery of organ weights (Table I).

Free ascorbic acid (AA):

An overall decrease in the levels of free ascorbic acid in testis and epididymides was noted by drugs except for an
increase (P 0.02) in caput by butabarbitone (15 days). By CPZ + AA treatment for 15 days an increase (P < 0.001) was observed in the levels of free ascorbic acid in all the three organs (Fig. 1).

Ascorbigen (ASG):

ASG by and large decreased or was not detectable in testis and cauda epididymis by the administration of all these drugs, whereas in caput, ASG was comparatively less affected. CPZ + AA administration for 7 days restored the levels of ASG in testis and cauda in comparison to those of CPZ treated rats. In the caput, recovery was noted by 15 days (Fig. 1).

Ascorbic acid utilization (AAU):

Compared to those of the testis and caput, the rate of AAU decreased by all three drugs. On the whole, CPZ brought about a decline, whereas an enhanced utilization (P < 0.05, 0.01) was noted in the testis and caput by chlordiazepoxide (7 days) and butobarbitone (15 days). Administration of CPZ + AA for 7 days restored the rate of AAU only in epididymides, whereas by 15 days, an overall increase was noted in all the three organs (Fig. 1).

Ascorbic acid macromolecule complexing (AA-MM) rate:

The rate of AA-MM complex remained by and large same as in control or increased in testis and caput by the administration of these drugs. However, CPZ treatment brought-
about a significant ($P < 0.001$) reduction in cauda. Ascorbic acid administration to CPZ treated rats (group III) restored the rate in testis only by 15 days (Fig. 1).

**AA-FR special peroxidase:**

All the three drugs brought about a significant ($P < 0.001$) decrease in AA-FR special peroxidase activity of testis and epididymides. CPZ + AA administration brought about an overall recovery in the enzymic activity of all organs except for a decrease in that of the testis by 7 days (Fig. 1).

**Cholesterol:**

By administration of CPZ, cholesterol levels on the whole decreased, but increased by butobarbitone and not affected by chlordiazepoxide. The increase by butobarbitone treatment (15 days) was more pronounced ($P < 0.001$) in epididymides than in testis. CPZ + AA treatment caused restoration or increase in the levels of cholesterol (Table II).

**Succinate dehydrogenase (SDH):**

An overall decrease ($P < 0.001$) was noted in SDH activity in all the three organs by the administration of drugs. Combined treatments with CPZ + AA (7 and 15 days) and CPZ + TP (7 days) did not restore the enzymic activity in these organs except in cauda. The CPZ + AA treatment was more effective in restoring SDH activity than CPZ + TP (Fig. 2).
Alkaline phosphatase:

The drug treatment caused an overall decrease ($P < 0.001$) in the activity of alkaline phosphatase of testis and epididymides except that a significant ($P < 0.01, 0.05$) activation was observed in the testis by chlordiazepoxide (15 days) and butabarbital (7 days). CPZ + AA (7 days) restored the enzymic activity only in cauda and in all organs by 15 days. In the caput an increase beyond control level was noted. By CPZ + TP administration however, the activity decreased significantly ($P < 0.001$) in all the three organs as compared with those of the group II (Fig. 2).

Acid phosphatase:

The enzymic activity was by and large increased by the administration of CPZ, and chlordiazepoxide but in the testis of butabarbital treated rats (7 days), it was same as in control rats but decreased in other organs. The increase was significant ($P < 0.02$) in caput and cauda. CPZ + AA administration restored the enzymic activity in testis (7 days) and cauda (by both 7 and 15 days), whereas by CPZ + TP administration recovery was observed only in cauda (Fig. 2).

Protein:

Administration of the drugs did not much alter the protein levels of testis except for a significant decrease ($P < 0.001$) by CPZ (15 days). In caput the concentration of protein either remained same as in control or increased
In cauda an inconsistent decrease or increase in protein levels was observed by drug treatment. The combined treatments with CPZ + AA and CPZ + TP either restored or increased the protein levels in these organs (Fig. 2).

DISCUSSION

It is evident from the present study that the tranquili­izers as well as sedative drugs have a marked effect in altering the metabolism of the testis and epididymides. A decrease in the organ weights and activities of some androgen dependent enzymes suggest that drugs like barbiturates and chlorpromazine (CPZ) inhibit the hypothalamo-pituitary-gonadal axis (Saldanha et al., 1972; Beattie et al., 1973; Norman et al., 1973; Sindgi and Rao, 1975; Levitt, 1975; Rao and Sindgi, 1976) and thereby reduce testicular functions including steroidogenesis due to inhibition of 3-β hydroxy steroid dehydrogenase (Rao, personal communication). A decrease in testosterone synthesis is also evident by the accumulation of cholesterol, especially by butobarbitone and by the recovery and / or increase in levels of some androgen sensitive parameters of these organs by the administration of testosterone propionate along with CPZ and also of ascorbic acid + CPZ. A similar recovery in the androgen dependent metabolism of the testicular and epididymal spermatozoa and of the secretory activity of
accessory reproductive glands in drug + ascorbic acid treated rats was observed (Buch, 1976; Chinoy and Sheth, 1976). The biochemical and electron spin resonance (ESR) studies of these authors reveal that ascorbic acid has a beneficial role in drug treated animals for restoring testicular androgenicity via the enhanced formation of its free radical, monodehydro-ascorbic acid which is an important electron donor in androgen synthesis (Biswas and Deb, 1970; Deb et al., 1976; Datta and Sanyal, 1976). It is therefore evident that androgen levels were restored in the drug treated rats by direct administration of testosterone propionate (TP) or indirectly by the ascorbic acid (AA) administration in vivo. The latter treatment was by and large, more effective since AA is known to have a synergistic action with testosterone in increasing testicular germ cell maturation, anabolic activity and those of androgen sensitive enzymes (Kutsky, 1973; Chapter I).

It is known that the administration of drugs and androgen deprivation by castration and cyproterone acetate (CA) treatment induce an increase in histamine levels (Orr and Quay, 1975; Assaykeen and Thomas, 1965; Chapters I and II), concomitant with increased synthesis and utilization of L-ascorbic acid (Longenecker et al., 1940; Conney et al., 1961; Chinoy and Sheth, 1976; Buch, 1976). Ascorbic acid is channelled for the detoxification of histamine (Subramanian et al., 1973, 1974;
Nandi et al., 1974), since it has inhibitory effects on testicular functions (Ellis, 1970). It is probable that the enhanced mobilization of ascorbic acid in reproductive tissues of drug treated animals (Chinoy and Sheth, 1976; Buch, 1976; this study) is required to maintain their functions, similar to the requirement of AA for restoring the activities of drug metabolizing enzymes in phenobarbital treated vitamin C deficient guinea pigs (Zannoni et al., 1972). The foregoing observations are substantiated by the fact that scorbutic guinea pigs are more sensitive to actions of drugs and are unable to metabolize the drugs efficiently (Garrattini and Shore, 1962; Chapters VI A, B) compared to animals fed on a vitamin C fortified diet.

In conclusion, the present study reveals that: (i) all the three drugs used alter the metabolism of testis and epididymides, (ii) ascorbic acid has a beneficial and protective role in maintaining the testicular and epididymal metabolism in drug treated rats via its involvement in steroidogenesis and synergistic action with testosterone for restoration of normal androgenic status of testis. The present data also indicates that the drug induced metabolic alterations are transient and reversible by either TP or ascorbic acid administration but more effectively by the latter. A combined TP + AA treatment might probably be the most conductive for the metabolic recovery of androgen target organs in view of their synergistic action.
A biochemical investigation was carried out to study the effects of tranquilizers, chlorpromazine (CPZ), chloridiazepoxide and a sedative (butobarbitone) as well as combined CPZ + ascorbic acid (AA) and CPZ + testosterone propionate (TP) treatment on reproductive physiology of male albino rats. The results show that (i) all the three drugs used alter the metabolism of the testis, caput and cauda epididymides, (ii) ascorbic acid has a beneficial and protective role in restoring and maintaining the testicular and epididymal metabolism in drug treated rats through its involvement in steroidogenesis and synergistic action with testosterone, (iii) the drug induced alterations are transient and reversible by either TP or ascorbic acid administration. However, the effects were more pronounced by the administration of ascorbic acid.
REFERENCES


5. Chapter I. Synergistic action of testosterone and ascorbic acid for maintenance of epididymal functions.

6. Chapter II. Effects of an anti-androgen, cyproterone acetate on metabolism of testis and epididymides of male albino rats.


TABLE I

Weights (in gm) of the testis and epididymides in control chlorpromazine (CPZ), CPZ + ascorbic acid (AA), CPZ + testosterone propionate (TP), chlordiazepoxide and butabarbitone treated rats

<table>
<thead>
<tr>
<th>Organs</th>
<th>I Control</th>
<th>II CPZ</th>
<th>III CPZ+AA</th>
<th>IV CPZ+TP</th>
<th>V Chlordiazepoxide</th>
<th>VI Butabarbitone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>15 days</td>
<td>7 days</td>
<td>15 days</td>
<td>7 days</td>
<td>15 days</td>
</tr>
<tr>
<td>Testis</td>
<td>1.38±</td>
<td>1.30±</td>
<td>1.10±</td>
<td>1.01±</td>
<td>0.78±</td>
<td>1.03±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.17</td>
<td>0.001</td>
<td>0.07</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Caput</td>
<td>0.20±</td>
<td>0.14±</td>
<td>0.15±</td>
<td>0.10±</td>
<td>0.14±</td>
<td>0.14±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.03</td>
<td>0.002</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.15±</td>
<td>0.07±</td>
<td>0.08±</td>
<td>0.05±</td>
<td>0.10±</td>
<td>0.11±</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.004</td>
<td>0.004</td>
<td>0.009</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.
### TABLE II

Cholesterol concentration (mg %) of testis and epididymides of control, chlorpromazine (CPZ), CPZ + ascorbic acid (AA), chlordiazepoxide, and butabarbital treated rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Normal control</th>
<th>CPZ 7 days</th>
<th>CPZ 15 days</th>
<th>CPZ + AA 7 days</th>
<th>CPZ + AA 15 days</th>
<th>Chlordiazepoxide 7 days</th>
<th>Chlordiazepoxide 15 days</th>
<th>Butabarbital 7 days</th>
<th>Butabarbital 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>0.11± 0.01</td>
<td>0.06± 0.002</td>
<td>0.06± 0.01</td>
<td>0.07± 0.01</td>
<td>0.07± 0.02</td>
<td>0.12± 0.001</td>
<td>0.14± 0.03</td>
<td>0.13± 0.001</td>
<td>0.18± 0.01</td>
</tr>
<tr>
<td>Caput</td>
<td>0.10± 0.01</td>
<td>0.08± 0.01</td>
<td>0.09± 0.001</td>
<td>0.15± 0.01</td>
<td>0.16± 0.01</td>
<td>0.11± 0.01</td>
<td>0.16± 0.03</td>
<td>0.12± 0.001</td>
<td>0.33± 0.001</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.14± 0.03</td>
<td>0.12± 0.01</td>
<td>0.18± 0.03</td>
<td>0.16± 0.01</td>
<td>0.15± 0.02</td>
<td>0.13± 0.001</td>
<td>0.14± 0.02</td>
<td>0.12± 0.03</td>
<td>0.41± 0.001</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.
Fig. 1

The concentrations of free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) complexing and ascorbic acid free radical (AA-RR) forming special peroxidase activity in testis, caput and cauda epididymis of rats

ND = not detectable
1 = Normal control
2 = Chlorpromazine (CPZ) treated - 7 and 15 days
3 = Chlorpromazine (CPZ + ascorbic acid (AA) treated - 7 and 15 days
4 = Chlordiazepoxide treated - 7 and 15 days
5 = Butobarbitone treated - 7 and 15 days
Fig. 2

The activities of succinate dehydrogenase (SDH), alkaline, acid phosphatases and protein levels in testis, caput and cauda epididymis of rats
1 = Normal control
2 = Chlorpromazine (CPZ) treated - 7 and 15 days
3 = Chlorpromazine (CPZ) + ascorbic acid (AA) treated - 7 and 15 days
4 = Chlorpromazine (CPZ) + testosterone propionate (TP) treated - 7 days
5 = Chlordiazepoxide treated - 7 and 15 days
6 = Butobarbitone treated - 7 and 15 days
**SDH**

μg formazan formed/100 mg fr. tissue wt. / 1 hr

- **CONTROL**
- **CPZ + TP**
- 7 DAYS
- 15 DAYS

**ALKALINE PHOSPHATASES**

U/l

**ACID PHOSPHATASES**

U/l

**PROTEIN**

mg/100 mg fr. tissue wt.

- 2, 3, 4, 5, 6

- TESTIS

- 2, 3, 4, 5, 6

- CAPUT

- 1, 2, 3, 4, 5, 6

- CAUDA
EFFECTS OF DRUGS ON THE REPRODUCTIVE PHYSIOLOGY OF MALE ALBINO RATS

2. NON-NARCOTIC ANALGESICS

INTRODUCTION

Many drugs are known to affect reproductive functions and bring about either an enhancement or reduction of fertility (Levitt, 1975; Satoskar and Bhandarkar, 1975). Administration of the aspirin leads to testicular atrophy and inhibition of spermatogenesis, alteration in testicular and epididymal sperm metabolism (Boyd, 1968, 1970; Buch, 1976). However, Cendella and Crowthamel (1973) reported that aspirin increased the fertility in subfertile male mice. The inhibitory effects of aspirin on the ovarian compensatory hypertrophy in rats due to its anti-gonadotrophic effect have also been demonstrated (Goldman and Poppens, 1972). Although analgesic drugs are known to alter the metabolism of carbohydrates, proteins and fats (Garattini et al., 1969; Collier, 1969; Satoskar and Bhandarkar, 1975; Chircoy and Kshatriya, 1976), these effects are transient and by and large reversible, especially in the reproductive organs and spermatozoa (Sheth, 1976; Buch, 1976). In the light of the above mentioned data, the present study was undertaken to investigate the effects of some commonly
used analgesic drugs like acetylsalicylic acid (aspirin), analgin and metamizole (novalgin) on testicular and epididymal physiology of rats.

MATERIALS AND METHODS

Healthy adult male albino rats (Rattus norvegicus) were used for the experiments. They were maintained in an air conditioned animal house (Temperature 26±2°C with 14 hours day light) on a standard diet (Hindustan Lever Ltd., Bombay) and water ad libitum.

The three analgesic drugs used were as follows:

a. Acetyl salicylic acid (aspirin) pure powder, Nicholas of India Ltd., Bombay.

b. Analgin (analgin) pure powder, Hoechst Pharmaceuticals Ltd., Backbay Reclamation, Bombay.

c. Metamizole (novalgin) 0.5 gm/tablet, Hoechst Pharmaceuticals Ltd., Backbay Reclamation, Bombay.

A known quantity of each drug was dissolved in water of known volume so as to obtain a concentration of 800 µg/ml which was found to be the minimum effective dose in rats. The tablets were first powdered, dissolved in water and the clear aqueous layer was used for the experiments. The animals were given an intramuscular injection of the drug (800 µg/animal/day) for 7 and 15 days respectively. The animals were
divided into the following groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No of animals used</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Aspirin treated 7 and 15 days</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
<tr>
<td>III</td>
<td>Aspirin + ascorbic acid treated</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>The rats were treated with</td>
<td>12</td>
<td>8th</td>
</tr>
<tr>
<td></td>
<td>aspirin as in group II and in</td>
<td></td>
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<td></td>
<td>addition were fed ascorbic acid</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(AA) orally (100 mg/animal/day)</td>
<td></td>
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<tr>
<td></td>
<td>for 7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Aspirin + testosterone treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The rats were treated with</td>
<td>12</td>
<td>8th</td>
</tr>
<tr>
<td></td>
<td>aspirin as in group II and in</td>
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<tr>
<td></td>
<td>addition were given</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>intramuscular injections of TP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(200 μg/animal/day) for 7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Analgin treated rats 7 and 15</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
<tr>
<td>VI</td>
<td>Metamizole (novalgin) treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rats 7 and 15 days</td>
<td>24</td>
<td>8th and 16th</td>
</tr>
</tbody>
</table>

After the respective treatments the animals were autopsied by cervical dislocation. The testes, and Caput and cauda - the epididymis were excised, blotted free of blood, weighed, and used for biochemical studies. The methods
followed are described in detail in Chapter I.

Ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU) and ascorbic acid macromolecule (AA-MM) complexing: (Chinoy et al., 1974).

Ascorbic acid free radical (AA-FR) forming special peroxidase: (Chinoy, 1973).

Cholesterol: (Pearson et al., 1953).

Succinate dehydrogenase (SDH): (Kun and Abood, 1949).

Alkaline and acid phosphatases: (Belfield and Goldberg, 1971).

Protein: (Gornall et al., 1949).

A minimum of six replicates were done for each parameter and treatment. The results were analyzed using students 't' test.

Fertility test:

Fertility test was performed according to the method described in Chapter II. The left uterine horn received the cauda epididymal sperm suspension of aspirin treated rats, whereas, the right uterine horn the sperm suspension from aspirin + ascorbic acid treated rats. After 8 days, implantation sites in uterine horns were observed.

RESULTS

Organ weights:

No significant change was noted in the weights of testis and epididymides by the administration of all three
analgesics except for an increase ($P < 0.1$) in the weight of caput by analgin (7 and 15 days), of cauda by aspirin (7 days) and analgin (15 days). Aspirin + AA and aspirin + TP treatments restored the organ weights to almost control values (Table I).

**Free ascorbic acid (AA):**

The levels of free ascorbic acid were by and large similar to control levels or increased in testis and caput by the administration of drugs. In cauda however, aspirin brought about a decrease ($P < 0.03$), but the other two drugs elevated AA significantly ($P < 0.001$). Aspirin + AA treatment restored the levels of free ascorbic acid to nearly control values in all organs (Fig. 1).

**Ascorbigen (ASG):**

ASG was on the whole decreased or not detectable in all three organs throughout the experimental period except for a significant ($P < 0.05$) increase in the caput by analgin and novalgin (7 days) and in the testis by aspirin (15 days, $P < 0.001$). In aspirin + AA treated rats, ASG was not detectable in the testis and caput whereas in cauda a recovery was noted in comparison with those of aspirin treated animals (Fig. 1).
Ascorbic acid utilization (AAU):

Administration of the drugs brought about an overall increase in the rate of AAU in all organs (P < 0.01) except in cauda wherein aspirin (15 days) caused a significant (P < 0.001) decrease. Treatment with aspirin + AA restored the normal rate of utilization in testis whereas, an increase (P < 0.001) was noted in cauda and caput (P < 0.1) (Fig. 1).

Ascorbic acid macromolecule (AA-MM) complexing rate:

The AA-MM complexing rate either remained unchanged or increased in testis by the administration of all the three analgesics. In the caput, the rate was same as in control or decreased significantly (P < 0.001) by aspirin (7 days) or increased by analgin (7 days). AA-MM complexing rate was not detectable by 15 days analgin treatment. In cauda the rate was either undetectable or decreased throughout the study except for a significant (P < 0.001; 0.01) increase by analgin and novalgin (7 days) (Fig. 1).

Aspirin + AA treatment restored the rate in testis and caput but in cauda, the AA-MM complex was undetectable (Fig. 1).

Ascorbic acid free radical (AA-FR) forming special peroxidase:

By the administration of all three analgesic drugs an overall decrease (P < 0.02) was noted in AA-FR special peroxidase activity in all the organs. By aspirin + AA administration, the enzymic activity was maintained at almost
the same level as in aspirin treated animals (Fig. 1).

**Cholesterol:**

An overall decrease \( P < 0.01 \) was observed in the testis by the administration of all analgesic drugs. In epididymides, cholesterol increased except for a decline in cauda by analgin (7 and 15 days) and novalgin (7 days). Aspirin + ascorbic acid administration restored the levels in the testis and caput but enhanced it in cauda (Table II).

**Succinate dehydrogenase (SDH):**

An overall decrease \( P < 0.001 \) in SDH activity was noted in testis and epididymides of drug treated rats. The decrease was significant \( P < 0.001 \) by analgin. Aspirin + AA and aspirin + TP administrations restored the activity in epididymides but not in the testis. The latter treatment was less effective than the former (Fig. 2).

**Alkaline phosphatase:**

By the administration of all the three analgesic drugs an overall decrease \( P < 0.02 \) was brought about in the enzymic activity in the testis and caput except that an increase was noted in testis by novalgin treatment (15 days). In cauda, aspirin administration did not alter the enzymic activity but analgin decreased it and novalgin administration activated the enzyme significantly \( P < 0.001 \). Aspirin + AA
treatment restored the enzymic activity in testis as compared to those of group II. In cauda the enzyme was activated beyond control levels but in caput it decreased. The combined treatment of aspirin + TP brought about a more pronounced recovery in the enzymic levels in the testis and caput than that by aspirin + AA treatment. However in cauda of group I\* rats, the enzyme did not recover (Fig. 2).

**Acid phosphatase:**

The enzymic activity remained by and large same as in control or decreased in drug treated animals except that an increase ($P < 0.001$, $0.01$) was brought about in the testis, caput and cauda by novalgin (7 days), in cauda by aspirin (15 days). The enzyme decreased by aspirin + AA administration in all three organs whereas aspirin + TP activated the enzyme in testis and restored it in cauda but not in caput (Fig. 2).

**Protein:**

By the administration of aspirin and analgin, the levels of protein were either decreased or remained almost same as in control in the testis and epididymides. However, a significant ($P < 0.001$) increase was noted in all three organs by novalgin treatment (7 and 15 days) and also in caput by aspirin (7 days). The combined treatment with aspirin + AA brought about a decrease in protein levels in testis, a significant increase ($P < 0.001$) in caput and restoration in
cauda. Aspirin + TP treatment restored the levels in testis to control values, elevated it in caput but decreased it in cauda (Fig. 2).

**Fertility test:**

Fertility test showed absence of implantation sites in either uterine horn.

**DISCUSSION**

The effects of the three analgesic drugs used viz., acetylsalicylic acid, metamizole, and analgin were comparable with the tranquilizers/sedatives in altering the physiology of the testis and epididymides (Chapter V A), and indicate a reduced androgenicity of the testis and androgen levels. Analgesic drugs like aspirin are known to cause a significant decrease in plasma levels of LH and testosterone (Clouet, 1971) and therefore cause impaired androgenesis by testis. The foregoing observations are substantiated by the recovery and/or increase in some of the androgen dependent parameters of the testis and epididymides by the administration of testosterone propionate (TP) along with aspirin. It is also probable that the overall decrease in metabolism of testis and epididymis including steroidogenesis is due to alterations in mitochondrial function, reduced glycolysis, uncoupling of oxidative phosphorylation and the low rate of energy production by analgesic drugs (Garattini et al., 1969; Wehman et al.,
The recovery in androgen dependent biochemical constituents of testis and epididymis by administration of aspirin + ascorbic acid (AA) reveals: (i) the restoration of testicular androgen synthesis and (ii) increased rate of tissue metabolism due to the availability and enhanced formation of AA free radical, monodehydroascorbic acid (MDHA), which is a powerful electron donor in oxido-reduction reactions including steroidogenesis (Fig. 3) (Biswas and Deb, 1970; Carballalva et al., 1974; Datta and Sanyal, 1976; Deb et al., 1976; Buck, 1976; Sheth, 1976). It is suggested that the recovery in testis and epididymal metabolism might also be related to a direct action of AA on enzymes (Harrer and King, 1941; Sebrell and Harris, 1967; Kutsky, 1973) and its synergism with pituitary LH and testosterone (Biswas, 1969; Kutsky, 1973; Chapters I and II). The enhanced utilization of ascorbic acid and MDHA in the testis and epididymides of analgesic drug treated rats could also be channelled for the detoxification of drug induced histamine (Abrahamian et al., 1973, 1974; Nandi et al., 1974) which otherwise impairs testicular functions (Ellis, 1970). Selye (1950) suggested that increased ascorbic acid utilization indicates adaptation of an organ to a particular stress condition. It is therefore evident from the results that cauda epididymis with its comparatively low rate of AA turnover is not quite adapted to overcome the...
drug induced stress in comparison to testis and caput epididymis. Moreover, the effects of aspirin were more pronounced than by the other two analgesic drugs (novalgin and analgin). The negative fertility test as evidenced by the absence of implantation sites in normal female rats inseminated with cauda epididymal sperm suspension of aspirin and aspirin + ascorbic acid treated rats, suggests that drugs like aspirin markedly affect the fertility of the males in agreement with the observations of others (Cenedella and Crouthamel, 1973; Buch, 1976). The fertility is not restored by drug + ascorbic acid treatment although the overall effects of ascorbic acid administration were more pronounced for restoration of epididymal and testicular metabolism than that of TP administration except for recovery of alkaline and acid phosphatases wherein TP was more effective.

**SUMMARY**

The effects of administration of analgesic drugs (acetyl salicylic acid, analgin and metamizole) on the metabolism of testis and epididymides of albino rats were studied. All the three drugs had more or less similar effects in altering some of the androgen sensitive parameters of these organs. The metabolic alterations were transient and reversible by exogenous administration of ascorbic acid or TP but fertility was not restored. On the whole, the ascorbic acid was more effective for recovery of the physiological integrity of testis and epididymis similar to those of rats treated with tranquilizers.
REFERENCES


8. Chapter I. Synergistic action of testosterone and ascorbic acid for maintenance of epididymal functions.
9. Chapter II. Effects of anti-androgen, cyproterone acetate on the metabolism of testis and epididymides of male albino rats.


Weights (in gm) of testis and epididymides of control, aspirin, aspirin + ascorbic acid (AA), aspirin + testosterone propionate (TP), analgin and novalgin treated rats

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>Aspirin</th>
<th>Aspirin+AA</th>
<th>Aspirin+TP</th>
<th>Analgin</th>
<th>Novalgin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 days</td>
<td>15 days</td>
<td>7 days</td>
<td>7 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Testis</td>
<td>1.38±</td>
<td>1.42±</td>
<td>1.41±</td>
<td>1.34±</td>
<td>1.10±</td>
<td>1.34±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.007</td>
<td>0.001</td>
<td>0.02</td>
<td>0.15</td>
<td>0.007</td>
</tr>
<tr>
<td>Caput</td>
<td>0.20±</td>
<td>0.19±</td>
<td>0.23±</td>
<td>0.22±</td>
<td>0.21±</td>
<td>0.26±</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.017</td>
<td>0.01</td>
<td>0.007</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.15±</td>
<td>0.15±</td>
<td>0.20±</td>
<td>0.17±</td>
<td>0.18±</td>
<td>0.18±</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.01</td>
<td>0.002</td>
<td>0.005</td>
<td>0.009</td>
<td>0.007</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
The concentration of cholesterol (mg %) in the testis and epididymides of control, aspirin, aspirin + AA, aspirin + TP, analgin and novalgin treated rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>Aspirin 7 days</th>
<th>Aspirin 15 days</th>
<th>Aspirin + AA 7 days</th>
<th>Aspirin + AA 15 days</th>
<th>Analgin 7 days</th>
<th>Analgin 15 days</th>
<th>Novalgin 7 days</th>
<th>Novalgin 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>0.11±</td>
<td>0.06±</td>
<td>0.08±</td>
<td>0.08±</td>
<td>0.05±</td>
<td>0.07±</td>
<td>0.08±</td>
<td>0.09±</td>
<td>0.005±</td>
</tr>
<tr>
<td>Caput</td>
<td>0.10±</td>
<td>0.12±</td>
<td>0.20±</td>
<td>0.11±</td>
<td>0.13±</td>
<td>0.15±</td>
<td>0.12±</td>
<td>0.19±</td>
<td>0.005±</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.14±</td>
<td>0.18±</td>
<td>0.18±</td>
<td>0.31±</td>
<td>0.09±</td>
<td>0.12±</td>
<td>0.11±</td>
<td>0.13±</td>
<td>0.001±</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.07</td>
<td>0.01</td>
<td>0.006</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
The concentrations of free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) complexing and ascorbic acid free radical (AA-FR) forming special peroxidase activity in testis, caput and cauda epididymis of rats

ND = not detectable
1 = Normal control
2 = Aspirin treated - 7 and 15 days
3 = Aspirin + ascorbic acid treated - 7 days
4 = Analgin treated - 7 and 15 days
5 = Metamizole treated - 7 and 15 days
Fig. 2

The activities of succinate dehydrogenase (SDH), alkaline, acid phosphatases and protein levels in testis, caput and cauda epididymis of rats

1 = Normal control
2 = Aspirin treated - 7 and 15 days
3 = Aspirin + ascorbic acid (AA) treated - 7 days
4 = Aspirin + testosterone propionate (TP) treated - 7 days
5 = Analgin treated - 7 and 15 days
6 = Novalgin treated - 7 and 15 days
Fig. 3

Metabolic turnover of ascorbic acid and the role of its free radical, monodehydroascorbic acid in detoxification mechanisms, steroidogenesis and other oxidoreduction reactions
FIG. 3 SHOWING THE INTER-RELATIONSHIPS IN THE METABOLISM OF ASCORBIC ACID, PROSTAGLANDINS AND C-AMP IN REPRODUCTIVE ORGANS.

- **PG**: Prostaglandins
- **AA**: Ascorbic Acid
- **IHA**: Dehydroascorbic Acid (DHA)
- **AA°**: Ascobic Acid
- **TC**: Charge Transfer Complex
- **MM**: Macromolecule Complex

**Key Processes**:
- **LH Secretion**: Stimulates Steroidogenesis in Testis, Epididymis, Ovary and Adrenals.
- **Adenyl Cyclase**: Stimulates Testosterone Biosynthesis.
- **Cyclic AMP (C-AMP)**: Used in detoxification mechanisms.
- **Increased Mitosis**: Stimulates biosynthetic reactions in other tissues.
- **LH and FSH**: Stimulates Leydig Cells of Testis.
- **Adenyl Cyclase**: Controls response of Leydig cells to LH and FSH.
- **STIMULATES**: Zucker, PEB, and PEBL.

**Miscellaneous**:
- **DETOXIFICATION MECHANISMS**: Stimulates LH secretion.
- **CHOLESTEROL TCA CYCLE**: Oxidation cycle.
- **ADENYL CYCLASE**: STIMULATES TESTOSTERONE BIOSYNTHESIS.

**Note**:
- The diagram illustrates the complex interplay between ascorbic acid, prostaglandins, and cyclic AMP in reproductive organs.
CHAPTER V C

EFFECTS OF DRUGS ON THE REPRODUCTIVE PHYSIOLOGY OF MALE ALBINO RATS

3. NARCOTIC ANALGESICS

INTRODUCTION

The narcotic analgesic drugs have both sedative and analgesic properties. Despite the fact that opiate narcotics and their derivatives like morphine, heroin and codeine have high potentiality for abuse by human beings, very little is known about their effects on reproductive functions. Some investigators (Baraclough and Sawyer, 1955; Hohlweg et al., 1961; Clouet, 1971; Dombrosky et al., 1973; Satoskar and Bhandarkar, 1975; Cicero et al., 1974; Chinoy and Sheth, 1977; Buch, 1976; Vyas and Singh, 1976) have reported that morphine/opium inhibits gonadotrophin secretion and ovulation, causes amenorrhea and sterility in the females, whereas, in males these drugs reduce plasma levels of the testosterone, affect metabolism of testis and epididymis, their spermatozoa and sex accessory organs resulting in a condition similar to castration. The metabolic significance of ascorbic acid for the maintenance of the physiological integrity of the testis and epididymis in rats treated with tranquilizers and
analgesic drugs has been elucidated in Chapters V A, B. It was therefore thought worthwhile to investigate the effects of opium and its major component morphine on male reproductive functions of albino rats with special emphasis on the turnover of ascorbic acid.

MATERIALS AND METHODS

Healthy adult male albino rats (Rattus norvegicus) weighing between 200-250 gm were used for all the experiments. They were maintained on standard diet (Hindustan Lever Ltd., Bombay) and water ad libitum in an air conditioned animal house (26±2°C) with 14 day light hours.

The drugs used are as follows:

a. Morphine: Morphine sulphate I.P. 10 mg/ml. Alembic Chemical Works Co. Ltd., Alembic Road, Baroda.


A known amount of pure opium was extracted in a known volume of water. The extract was filtered, tested for alkaloids with Dragendorff's reagent (Sarkar and Rakshit, 1960) and the volume was made up to obtain a concentration of 1 mg/ml. Both opium and morphine were injected intramuscularly (1 mg/animal/day) for 7 days. The animals were divided into the following groups.
Group Treatment No. of animals used Day of autopsy
---
I Normal control 12
II Opium treatment 7 days 12 8th
III Morphine treatment 7 days 12 8th
IV Morphine + ascorbic acid treatment (AA) 12 8th
Morphine was injected as in group III (1 mg/animal/day) and in addition ascorbic acid was given orally (100 mg/animal/day) continuously for 7 days

After the respective treatments, the animals were sacrificed by cervical dislocation. The testis and epididymides were excised, blotted free of blood, weighed and utilized for biochemical studies according to the methods described in detail in Chapter I.

Ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complexing rate (AA-MM): (Chinoy et al., 1974).

Ascorbic acid free radical (AA-FR) forming special peroxidase: (Chinoy, 1973).

Cholesterol: (Pearson et al., 1953).

Succinate dehydrogenase (SDH): (Kun and Abood, 1949).
Alkaline and acid phosphatases: (Belfield and Goldberg, 1971).
Protein: (Gornall et al., 1949).

A minimum of six replicates were done for each parameter and tissue. The results were analyzed statistically by student's 't' test.

RESULTS

Organ weights:

No significant changes were noted in the weights of testis and epididymides by drug treatment as well as by treatment with drug+ ascorbic acid (AA) (Table I).

Free ascorbic acid (AA):

Administration of opium and morphine brought about a significant ($P < 0.01$) increase in the levels of free AA in caput and cauda, whereas in the testis not much alteration was noted (Fig. 1).

Ascorbigen (ASG):

The levels of ASG were not detectable by opium in the testis and by both drugs in caput. Administration of morphine significantly ($P < 0.02$) elevated the concentration in the testis. In cauda, ASG decreased by both the drugs (Fig. 1).

Ascorbic acid utilization (AAU):

By treatments with opium and morphine the rate of utilization was increased in all organs. The increase was significant ($P < 0.01$) in caput and cauda (Fig. 1).
Ascorbic acid macromolecule (AA-MK) complexing rate:

There was an overall increase in the rate of AA-MM complex in all these organs except for a decrease ($P < 0.05$) in cauda by morphine administration (Fig. 1).

Ascorbic acid free radical (AA-FR) forming special peroxidase:

An overall significant ($P < 0.001$) decrease was noted in the activity of AA-FR special peroxidase in all the three organs by morphine and opium administration (Fig. 1).

Succinate dehydrogenase (SDH):

SDH activity decreased ($P < 0.1$) by both the drugs in all three organs. By ascorbic acid administration to morphine treated animals, a recovery in enzymic activity was observed only in the cauda (Fig. 2).

Alkaline phosphatase:

An overall significant ($P < 0.001$) decrease was observed in alkaline phosphatase activity in all the three organs after opium and morphine treatments except in cauda of morphine treated rats.

The enzymic activity recovered in the testis and epididymides of morphine + ascorbic acid (AA) treated rats in comparison with those of group III. The recovery was significant in testis ($P < 0.01$) (Fig. 2).
Acid phosphatase:

By opium administration, the enzymic activity was reduced significantly ($P < 0.001$) in all the three organs. On the other hand, morphine treatment did not alter the acid phosphatase activity in the testis and caput but activated it in the cauda. Morphine + AA administration also activated the enzyme in the testis and cauda but not in caput (Fig. 2).

DISCUSSION

The effects of opium on the metabolism of testis and epididymides were observed to be more pronounced than those of morphine unlike in the accessory sex organs (Sheth, 1976). However, both the drugs decreased some of the androgen dependent enzymes (SDH, alkaline phosphatase and AA-PR special peroxidase) of the testis and epididymis probably due to the morphine induced androgen deprivation which is similar to that seen following castration. As a consequence of the inhibitory effects of opiates on pituitary-gonadal functions, decreased libido and sterility occur in treated animals (Clouet, 1971; Dombrosky et al., 1973; Cicero et al., 1974; Unpublished observations; Satoskar and Bhandarkar, 1975; Buch, 1976; Chinoy and Sheth, 1977; Bach, 1977). The decreased androgenicity as induced by opiates could be correlated with their capacity to mimic steroid hormones and displace testosterone at receptor sites (La Bella, 1975).
A significant increase of epididymal acid phosphatase in morphine treated rats might be correlated with disposal of non-motile sperms (Buch, 1976). A similar increase in lysosome bodies in heroin addicts (diacetyl morphine) have been observed by Bhakthan (1973).

The opiate narcotics caused an increased mobilization and utilization of the bound form of ascorbic acid (ascorbigen) in conformity with the work of others (Clouet, 1971; Borrell and Borrell, 1975; Borrell et al., 1975). Tranquilizers, sedatives and non-narcotic analgesic drugs were also found to increase the utilization of ascorbic acid in testis and epididymides (Chapters V A, B) which is needed for detoxification of morphine-induced histamine (Subramanian et al., 1974; Satoskar and Bhandarkar, 1975). The reproductive organs are known to have high amounts of histamine in androgen deprived conditions, especially in the rats (Assaykeen and Thomas, 1965; Orr and Quay, 1975). The enhanced ascorbic acid utilization therefore is probably a protective mechanism against the harmful effects of histamine on testis and epididymis (Chapters V A, B; Ellis, 1970). The combined treatments of morphine + ascorbic acid were found to have a beneficial effect in restoring testicular androgenicity and thereby maintaining the functional integrity of epididymis in drug treated rats by its involvement in testicular androgenesis (Biswas and Deb, 1970). The opiate
induced alterations in the testis and epididymides are therefore by and large reversible, similar to those of analgesic and tranquilizer drug induced changes.

SUMMARY

A study on the effects of narcotic analgesic drugs (Opium and morphine) on the testicular and epididymal physiology of rats was carried out. Opium treatment manifested more pronounced influence than morphine. The narcotic induced alterations were by and large reversible by ascorbic acid administration.
REFERENCES


8. Chapter I. Synergistic action of testosterone and ascorbic acid for maintenance of epididymal functions.


TABLE I

Weights (in gm) of testis and epididymides of control, opium, morphine and morphine + ascorbic acid (AA) treated rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control (7 days)</th>
<th>Opium (7 days)</th>
<th>Morphine (7 days)</th>
<th>Morphine + AA (7 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>1.38± 0.01</td>
<td>1.25± 0.01</td>
<td>1.29± 0.07</td>
<td>1.27± 0.09</td>
</tr>
<tr>
<td>Caput</td>
<td>0.20± 0.01</td>
<td>0.22± 0.02</td>
<td>0.18± 0.007</td>
<td>0.21± 0.02</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.15± 0.02</td>
<td>0.18± 0.01</td>
<td>0.14± 0.007</td>
<td>0.18± 0.02</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
The concentrations of free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) complexing and ascorbic acid free radical (AA-FR) forming special peroxidase activity in testis, caput and cauda epididymis.

ND = not detectable

1 = Normal control
2 = Opium treated - 7 days
3 = Morphine treated - 7 days
Fig. 2

The activities of succinate dehydrogenase (SDH), alkaline and acid phosphatases, in testis, caput and cauda epididymis of rats
1 = Normal control
2 = Opium treated - 7 days
3 = Morphine treated - 7 days
4 = Morphine + ascorbic acid (AA) treated - 7 days
CHAPTER V D

EFFECTS OF DRUGS ON THE REPRODUCTIVE PHYSIOLOGY OF MALE ALBINO RATS

4. CHOLINERGIC DRUGS

INTRODUCTION

Nicotine, a major alkaloid of tobacco causes peripheral vasoconstriction and affects reproductive functions (Levitt, 1975; Satoskar and Bhandarkar, 1975; Abrol, personal communication), so that smokers run the risk of becoming sterile. The present study was therefore undertaken to investigate the effects of nicotine and tobacco on the testis and epididymal physiology with special reference to the role of ascorbic acid in drug treated rats.

MATERIALS AND METHODS

Healthy adult male albino rats (200-250 gm) were used for the experiments. They were maintained on a supply of standard diet (Hindustan Lever Ltd., Bombay) and water ad libitum, in an air conditioned animal house (26±2°C) with 14 day light hours.

The drugs used were as follows:

Tobacco (Wills filtered) was boiled in water and filtered. The extract was tested for the presence of alkaloids
with Dragondorff's reagent (Sarkar and Rakshit, 1960).

Nicotine was dissolved in water as it is miscible with water below 60°C and above 210°C (Sarkar and Rakshit, 1960). Both the drugs were injected intramuscularly at a concentration of 1 mg/ml for 7 days. The animals were grouped as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals used</th>
<th>Day of autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Nicotine treated 7 days</td>
<td>12</td>
<td>8th</td>
</tr>
<tr>
<td>III</td>
<td>Tobacco treated 7 days</td>
<td>12</td>
<td>8th</td>
</tr>
<tr>
<td>IV</td>
<td>Tobacco+ascorbic acid (AA) treated rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The rats were treated with tobacco as in group III (1 mg/animal/day) and were given ascorbic acid orally (100 mg/day/animal) continuously for 7 days</td>
<td>12</td>
<td>8th</td>
</tr>
<tr>
<td>V</td>
<td>Tobacco+testosterone propionate (TP) treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The animals were injected tobacco as in group II and in addition were given intramuscular injections of TP (200 μg/animal/day) for 7 days</td>
<td>12</td>
<td>8th</td>
</tr>
</tbody>
</table>
The animals were autopsied after the respective treatments, their testis and epididymides excised, blotted free of blood, weighed and utilized for biochemical estimations of the following parameters by the methods described earlier in Chapter I.

- Ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complexing: (Chinoy et al., 1974).
- Ascorbic acid free radical forming special peroxidase: (Chinoy, 1973).
- Cholesterol: (Pearson et al., 1953).
- Succinate dehydrogenase (SDH): (Kun and Abood, 1949).
- Alkaline and acid phosphatases: (Belfield and Goldberg, 1971).
- Protein: (Gornall et al., 1949).

A minimum of six replicates were done for each parameter and tissue and the result were analyzed by using student's 't' test.

**RESULTS**

**Organ weights:**

No significant changes were observed in the weights of testis and epididymides by the administration of tobacco and nicotine except for a decrease ($P < 0.05$) by tobacco + AA (Table I).
Free ascorbic acid (AA):
The free AA levels were significantly decreased \( (P < 0.001) \) by the administration of tobacco, whereas, by nicotine and tobacco + AA treatments, an overall increase in the levels of free AA was noted throughout the study (Fig. 1).

Ascorbigen (ASG):
ASG by and large increased significantly \( (P < 0.001) \) in testis and caput by both the drugs but in cauda it decreased. By tobacco + AA administration, ASG was not detectable in all the organs (Fig. 1).

Ascorbic acid utilization (AAU):
Nicotine administration caused an overall increase in AAU whereas, by tobacco a decrease was observed which was quite significant \( (P < 0.01) \) in cauda (Fig. 1).

Ascorbic acid macromolecule complexing rate (AA-MM):
The rate of AA-MM complex decreased in cauda by both drugs and in the testis by tobacco. On the contrary, in the caput, the rate was enhanced by nicotine and remained same as in the normal control by tobacco treatment. The combined treatment with tobacco + AA restored the rate of AA-MM complex to control level only in cauda (Fig. 1).
Ascorbic acid free radical (AA-FR) forming special peroxidase:

AA-FR special peroxidase activity was reduced significantly \( (P < 0.05) \) in all the three organs by the administration of tobacco and nicotine. Tobacco + AA treatment was found to bring about a recovery in the enzymic activity in all organs as compared to tobacco treatment. The recovery was significant \( (P < 0.01) \) in testis (Fig. 1).

Cholesterol:

Concentration of cholesterol was significantly \( (P < 0.001) \) increased by the administration of nicotine and tobacco in all the tissues except that the latter drug did not affect cholesterol level in the testis. Tobacco + AA treatment elevated the levels in caput and restored it in cauda in comparison to those of tobacco treatment. But cholesterol levels were not altered in the testis (Table II).

Succinate dehydrogenase (SDH):

Nicotine administration did not alter SDH activity in epididymides whereas in the testis, it decreased. However, tobacco treatment caused a significant \( (P < 0.05) \) decrease in all organs. The combined treatment with tobacco + AA was effective in restoring SDH levels only in caput, whereas, by tobacco + TP administration, restoration of enzymic activity was noted only in cauda (Fig. 2).
Alkaline phosphatase:
An overall significant ($P < 0.001$) decrease was observed in alkaline phosphatase activity in all three organs by the administration of both drugs. Tobacco + AA administration however restored the enzymic activity in all organs. By tobacco + TP administration, the recovery was noted only in caput, while in the testis and cauda, the enzymic activity was significantly ($P < 0.001$) reduced in comparison to those of the other groups (Fig. 2).

Acid phosphatase:
Acid phosphatase activity decreased significantly ($P < 0.001$) in all the organs by the administration of tobacco and nicotine. Treatments with tobacco + AA and tobacco + TP activated ($P < 0.001$) the enzyme in testis and restored it in cauda and caput (Fig. 2).

Protein:
The protein levels were not much altered by nicotine except that a significant ($P < 0.001$) increase was noted in caput. On the other hand, tobacco treatment brought about a decrease ($P < 0.001$) in the protein of the testis and caput, but an increase in the cauda. By tobacco + AA treatment, protein concentration recovered in caput and by tobacco + TP administration it increased significantly ($P < 0.001$) in caput beyond control value (Fig. 2).
DISCUSSION

The results reveal that the effects of tobacco were more pronounced than those of nicotine in reducing some androgen sensitive parameters. This might be attributed to the alterations in the plasma levels of LH (Blake et al., 1972, 1974), which would affect testicular androgenesis. The decrease in SDH, a mitochondrial androgen-sensitive enzyme by administration of cholinergic drugs could thus be related to the androgen deprived condition or else the drug-induced structural changes in mitochondria (Gariola and Aleem, 1973). The recovery of androgen dependent parameters by treatment of rats with tobacco + testosterone propionate supports the view that reduced androgen levels occur in cholinergic drug treated rats.

Nicotine was found to enhance the overall ascorbic acid (AA) metabolism, while tobacco brought about a decrease. An increased AA turnover is indicative of response of an organ to a particular stress condition (Selye, 1950). It is therefore evident that the reproductive organs were able to overcome the nicotine-induced stress more effectively than that imposed by tobacco, due to the greater retention of ascorbic acid in the body after nicotine treatment (Keith and Pelletier, 1973; Pelletier, 1968, 1970). The tissue storage of ascorbic acid is a protective action against acetaldehyde.
toxicity in smokers and alcoholics (Prince et al., 1975). The recovery of some androgen sensitive parameters of testis and epididymis in drug and ascorbic acid (AA) treated rats supports the hypothesis that ascorbic acid plays a protective role in restoring and maintaining their metabolism and the secretory activity of accessory organs (Chinoy and Sheth, 1977), via the formation of its free radical, monodehydro-ascorbic acid, which participates in testicular steroidogenesis (Biswa and Deb, 1970; Carballeiva et al., 1974), and thereby restores androgen levels. It is also likely that the increase in androgen sensitive enzymes in the epididymides and testis of animals of group IV, is due to their direct activation by ascorbic acid or else its synergistic action with testosterone (Harrer and King, 1971; Sebrell and Harris, 1967; Kutsky, 1973; Chapter I).

**SUMMARY**

The testicular and epididymal physiology in nicotine and tobacco treated rats revealed a more pronounced inhibitory effect of tobacco in altering the levels of androgen sensitive parameters, concomitant with decreased ascorbic acid utilization. Recovery to the normal metabolic turnover pattern was obtained by ascorbic acid or testosterone propionate (TP) administration. However, ascorbic acid was more effective than TP in overcoming the adverse effects of the drugs.
REFERENCES

1. Abrol, B.M., personal communication.


7. Chapter I. Synergistic action of testosterone and ascorbic acid for maintenance of epididymal functions.


**TABLE I**

Weights (in g) of testis and epididymides in control, nicotine, tobacco, tobacco + ascorbic acid (AA) and tobacco + testosterone propionate (TP) treated rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>Nicotine 7 days</th>
<th>Tobacco 7 days</th>
<th>Tobacco + AA 7 days</th>
<th>Tobacco + TP 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>1.38± 0.01</td>
<td>1.35± 0.07</td>
<td>1.22± 0.13</td>
<td>0.86± 0.07</td>
<td>1.18± 0.29</td>
</tr>
<tr>
<td>Caput</td>
<td>0.20± 0.01</td>
<td>0.21± 0.02</td>
<td>0.24± 0.01</td>
<td>0.13± 0.01</td>
<td>0.21± 0.005</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.15± 0.02</td>
<td>0.17± 0.02</td>
<td>0.19± 0.002</td>
<td>0.11± 0.01</td>
<td>0.22± 0.017</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
TABLE II

Concentration of cholesterol (in mg %) in testis and epididymides of control, nicotine, tobacco and tobacco + ascorbic acid (AA) treated rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control 7 days</th>
<th>Nicotine 7 days</th>
<th>Tobacco 7 days</th>
<th>Tobacco + Ascorbic acid 7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis</td>
<td>0.11±0.01</td>
<td>0.30±0.01</td>
<td>0.12±0.005</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Caput</td>
<td>0.10±0.03</td>
<td>0.14±0.02</td>
<td>0.17±0.01</td>
<td>0.25±0.001</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.14±0.03</td>
<td>0.22±0.02</td>
<td>0.28±0.02</td>
<td>0.12±0.01</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
The concentrations of free ascorbic acid (AA), ascorbigen (ASG), rates of ascorbic acid utilization (AAU), ascorbic acid macromolecule (AA-MM) complexing and ascorbic acid free radical (AA-FR) forming special peroxidase activity in testis, caput and cauda epididymis of rats

ND = not detectable
1 = Normal control
2 = Nicotine treated - 7 days
3 = Tobacco treated - 7 days
4 = Tobacco + ascorbic acid (AA) treated - 7 days
Fig. 2

The activities of succinate dehydrogenase (SDH), alkaline, acid phosphatases and protein levels in testis, caput and cauda epididymis of rats

1 = Normal control
2 = Nicotine treated - 7 days
3 = Tobacco treated - 7 days
4 = Tobacco + ascorbic acid (AA) treated - 7 days
5 = Tobacco + testosterone propionate (TP) treated - 7 days